subject matter of the claims to enable one skilled in the pertinent art to make and use the claimed invention. Applicant is not required to enable that which is not claimed.

The Examiner, in item 21, stated that:

1. "[t]he specification is not enabled for a method of producing apomictic seeds…".
2. "[t]he specification does not actually demonstrate that plant cells transformed with the nucleotide sequence encoding SERK rendered the cell embryogenic…"
3. "[g]iven the breadth of the claims encompassing a method of producing apomictic seeds comprising transformation with **any** nucleotide sequence which renders a plant cell embryogenic or a method of obtaining embryonic cells or of producing somatic embryos comprising ectopically overexpressing the SERK gene…" Emphasis added.

Applicant has canceled all method claims. Therefore, the Examiner's first and third listed bases for the enablement rejection set forth immediately above are no longer applicable. In addition, Applicant's pending claims are directed to isolated genes and the proteins encoded thereby, not to **any** nucleotide sequences.

With regard to the second listed basis for the Examiner's rejection set forth in item 21, Applicant respectfully points out that the remaining independent claim 47 is directed to protein kinases having the amino acid sequences depicted in SEQ ID NOS. 3, 21, and 33 and that hybridize with isolated DNA having the sequences depicted in SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.20 or SEQ ID NO.32. Claim 47 does not claim the invention of rendering a plant cell embryogenic. Thus, Applicant respectfully submits that the Examiner's rejection under 35 USC 112 is not appropriate.

Clearly, these claimed nucleotide sequences and the products encoded thereby are fully disclosed in the sequence listing of the application, and how to make and use these sequences is described in detail in the application's Detailed Description of the Invention section. Furthermore, the methods needed to isolate the claimed sequences were well known to those skilled in the art, and the specification cites a number of publications that establish the artisans' high skill level and knowledge at the time of filing. See also, for example, pages 3-5 and pages 7-8.

The Examiner cited *Genentech v. Novo Nordisk*, 42 USPQ2d 1001 (Fed. Cir. 1997), for the proposition that the "unpredictability of the art and lack of guidance as discussed above, undue experimentation would be required by one skilled in the art to make and use the claimed invention." In Genentech, the specification merely contained generic statements stating the possibility of cleavable fusion expression, the hGH sequence, and a possible enzyme for cleaving hGH, without

otherwise enabling the method. In that case, the court stated that although the specification need not disclose that which is well known in the art, the lack of specific starting material or of the conditions under which a process can be carried out required undue experimentation. In significant contrast, the specification in the present case clearly sets forth the starting materials, conditions, and processes needed to make and use the claimed sequences and proteins encoded therefrom.

Applicant respectfully submits that, based on the specification and on the common knowledge of cloning and transformation techniques at the time the specification was filed, the claimed subject matter is enabled by the specification. Thus, in view of the amendments and above remarks, it is submitted that this application is now ready for allowance.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call Applicant's undersigned attorney.

Respectfully submitted,

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Version with markings to show changes made

In the specification:

The following sentence has been added immediately after the title of invention on page 1:
This application is a continuation of PCT/EP97/02443, which claims priority from European Application No. 01108901.8.

The following heading has been added following the title of the invention on page 1:
Field of the Invention

The following heading has been added following the first paragraph on page 1:
Background of the Invention

The following heading has been added following the second full paragraph on page 2 and immediately before the paragraph beginning with the words "According to the present invention:"
Summary

The third full paragraph on page 3 has been amended as follows:

Expression of the sequence may yield a protein kinase capable of spanning a plant cell membrane. Typically the kinase may be a leucine rich repeat receptor like kinase which has the capacity to auto-phosphorylate. ~~The skilled man~~ Those skilled in the art will recognize what is meant by the term "leucine rich repeat receptor like kinase". Examples of such proteins include *Arabidopsis* RLK5 (Walker, 1993), *Arabidopsis* RPS2 (Bent *et al.* 1994), Tomato *CF-9* gene product (Jones *et al.* 1994), Tomato N (Whitham *et al.* 1994), *Petunia* PRK1 (Mu *et al.* 1994), the product of the *Drosophila Toll* gene (Hashimoto *et al.* 1988), the protein kinase encoded by the rice *OsPK10* gene (Zhao *et al.* 1994), the translation product of the rice EST clone ric2976 and the product of the *Drosophila Pelle* gene (Shelton and Wasserman, 1993). Still further examples of such proteins include the TMK1, Clavatal, Erecta, and TMKL1 gene products from *Arabidopsis*, the Flightless-1 gene product from *Drosophila*, the TrkC gene product from pig, the rat LhCG receptor

and FSH receptor, the dog TSH receptor, and the human Trk receptor kinase. The protein may comprise a ligand binding domain, a proline box, a transmembrane domain, a kinase domain and a protein binding domain. In many receptor kinases the extracellular (ligand binding) domain serves as an inhibitor of the kinase domain in the ligand-free state. This arrest is removed after binding of the ligand. Accordingly, in one embodiment of the invention the protein either lacks a ligand binding domain or the domain is functionally inactivated so that the kinase domain can be constitutively active in the absence of an activating signal (ligand). Whether or not the protein possesses a ligand binding domain - functional or otherwise, once expressed and incorporated into the plant cell membrane the protein binding domain is preferably located intra-cellularly.

The last paragraph on page 5 has been amended as follows:

In particular, the invention embodies a DNA comprising a DNA sequence encoding a N-terminal protein fragment having the following amino acid sequence as depicted in SEQ ID No. 22, AAs 48-66: Gln Ser Trp Asp Pro Thr Leu Val Asn Pro Cys Thr Trp Phe His Val Thr Cys Asn.

The second paragraph on page 6 has been amended as follows:

In addition, non-conservative replacements may also occur at a low frequency. Accordingly, the invention futher embodies a DNA comprising a DNA sequence encoding a N-terminal protein fragment having the following amino acid sequence as depicted in SEQ ID No. 22, AAs 47-67: Val Xaa Gln Ser Trp Asp Pro Thr Leu Val Asn Pro Cys Thr Trp Phe His Val Thr Cys Asn, with Xaa being a variable amino acid, but preferably Leu or Val.

The third paragraph on page 6 has been amended as follows:

Especially preferred within the scope of the invention is a DNA comprising a DNA sequence encoding a N-terminal protein fragment having the following amino acid sequence as depicted in SEQ ID No. 23, AAs 47-97: Val Xaa Gln Ser Trp Asp Pro Thr Leu Val Asn Pro Cys Thr Trp Phe His Val Thr Cys Asn Xab Xac Xad Xae Val Xaf Arg Val Asp Leu Gly Asn Xag Xah Leu Ser Gly His Leu Xai Pro Glu Leu Gly Xaj Leu Xak Xal Leu Gln, with Xaa to Xak representing variable amino acids, but preferably

Xaa = Leu or Val

Xab = Asn or Gln

Xac = Glu or Asp or His

Xad = Asn or His

Xae = Ser or Arg or Gln

Xaf = Ile or Thr

Xag = Ala or Ser

Xah = Glu or Asn

Xai = Val or Ala

Xaj = Val or Lys

Xak = Lys or Glu

Xal = Asn or His

The last paragraph on page 9 has been amended as follows:

Parts of plants, such as for example flowers, stems, fruits, leaves, roots originating in transgenic plants or their progeny previously transformed by means of the process of the invention and therefore consisting at least in part of transgenic cells, are also an object of the present invention. Especially preferred are apomictic seeds.

The second paragraph on page 14 has been amended as follows:

Additional constructs are generated that have constitutive receptor kinase activity. Most of the receptor kinases of the SERK type act as homodimeric receptors, requiring autophosphorylation before being able to activate downstream signal transduction cascades. In many receptor kinases the extracellular domain serves as an inhibitor of the kinase domain in the ligand-free stage. This arrest is removed after binding of the ligand (Cadena and Gill, 1992). By introduction of a SERK construct, from which the extracellular ligand-binding domain has been removed, mutant homodimeric (in cells that do not have a natural population of SERK proteins) or heterodimeric (in cells that also express the unmodified forms) proteins can be generated with a constitutively activated kinase domain. This approach, when coupled to one of the promoters active in the nucellar region, results in activation of the embryogenic pathway in the absence of the activating signal. This may be an important alternative in cases where it is necessary or desirable to have activation of the SERK pathway only dependant on specific promoter activity and independent of temporal

regulation of an activating signal. Introduction of SERK constructs that result in fertilization-independent-embryogenesis (fie) are tested in other species for their effect. In order to recognize the fie phenotype, ~~the skilled man~~ those skilled in the art will use appropriate male sterile backgrounds. However, pollination is often necessary for apomixis of the adventitious embryony type, in order to ensure the production of endosperm.

The following heading has been added on page 16 immediately before the paragraph beginning with the words "Figure 1 shows the results:"

Brief Description of the Drawings

The following heading has been added on page 18 immediately before the paragraph beginning with the words "The following description:"

Detailed Description of the Invention

The second paragraph on page 18 has been amended as follows:

Labeled probes for differential screening were obtained from RNA out of a <30 mm sieved sub-population of cells from either embryogenic or non-embryogenic cell cultures. Employing these probes in a library screen of approximately 2000 plaques yielded 26 plaques that failed to show any hybridization to either probe. These so-called cold plaques were purified and used for further analysis. From the total number of plaques that did hybridize, about 30 did so only with the probe from embryogenic cells. ddRT-PCR reactions using a combination of one anchor primer and one decamer primer were performed on mRNA isolated from three embryogenic, and three non-embryogenic suspension cultures. About 50 different ddRT-PCR fragments were obtained from each reaction. Using combinations of three different anchor and six different decamer primers, a total of approximately 1000 different cDNA fragments was visualized. Six of these PCR fragments were only found in lanes made with mRNA from <30 mm populations of cells from embryogenic cultures (Table 1) and with oligo combinations of the anchor primer (5'-TTTTTTTTTTTGC-3') and the decamer primers (5'-GGGATCTAAG-3'), (5'-ACACGTGGTC-3'), (5'-TCAGCACAGG-3') (as depicted in SEQ ID Nos. 4, 5, 6, and 7, respectively). Because differential PCR fragments often consist of several unresolved cDNA fragments (Li *et al.* 1994), cloning proved to be essential prior to undertaking further characterization of the PCR fragments obtained.

The last paragraph on page 19 has been amended as follows:

cDNA clone 31-50 encodes a leucine-rich repeat containing receptor-like kinase

The mRNA corresponding to the isolated clone 31-50 had an open reading frame of 1659 nucleotides encoding a protein with a calculated Mw of 55 kDa. Because clone 31-50 is mainly expressed in embryogenic cell cultures it was renamed Somatic Embryogenesis Receptor Kinase (SERK). The SERK protein contains a N-terminal domain with a five-times repeated leucine-rich motif that is proposed to act as a protein-binding region in LRR receptor kinases (Kobe and Deisenhofer, 1994). Between the extracellular LRR domain of SERK and the membrane-spanning region is a 33 amino acid region rich in prolines (13), that is unique for the SERK protein. Of particular interest is the sequence SPPPP (SEQ ID No. 17), that is conserved in extensins, a class of universal plant cell wall proteins (Varner and Lin, 1989). The proposed intracellular domain of the protein contains the 11 subdomains characteristic of the catalytic core of protein kinases. The core sequences HRDVKAAN (SED ID No. 18) and GTLGYIAPE (SEQ ID No. 19) in respectively the kinase subdomains VB and VIII suggest a function as a serine / threonine kinase (Hanks *et al.* 1988). Another interesting feature of the intracellular part of the SERK protein is that the C-terminal 24 amino-acids resembles a single LRR. The serine and threonine residues present within the intracellular LRR sequence are surrounded by acidic residues and might be targets for the autophosphorylation of SERK, thereby regulating the ability of other proteins to interact with this receptor-kinase in a similar fashion as described for the SH2 domain of the EGF family of tyrosine receptor kinases.

The last paragraph on page 25 has been amended as follows:

Differential Display RT-PCR

Differential display of mRNA was performed essentially as described by Liang and Pardee (1992). cDNA synthesis took place by annealing 1 mg of total RNA in 10 ml buffer containing 200 mM KCl, 10 mM Tris-HCl (pH 8.3), and 1 mM EDTA with 100 ng of one of the following anchor primers: (5'-TTTTTTTTTTTGC-3'), (5'-TTTTTTTTTTTCTG-3'), (5'-TTTTTTTTTTTCA-3') (SEQ ID Nos. 4, 8, and 9, respectively). Annealing took place by heating the mix for 3 min. at 83°C followed by incubation for 30 min at 42°C. Annealing was followed by the addition of 15 ml pre-

warmed cDNA buffer containing 16 mM $MgCl_2$, 24 mM Tris-HCl (pH 8.3), 8 mM DTT, 400 mM dNTP, and 4 Units AMV reverse transcriptase (Gibco BRL). cDNA synthesis took place at 42°C for 90 min. First strand cDNA was phenol/chlorophorm extracted and precipitated with ethanol using glycogen as a carrier. The PCR reaction was performed in a reaction volume of 20 ml containing 10% of the synthesized cDNA, 100 ng of anchor primer, 20 ng of one of the following 10-mer primers: (5'-GGGATCTAAG-3') , (5'-TCAGCACAGG-3'), (5'-GACATCGTCC-3'), (5'-CCCTACTGGT-3'), (5'-ACACGTGGTC-3'), (5'-GGTGACTGTC-3') (SEQ ID Nos. 5, 7, 10, 11, 12, and 13, respectively), 2 mM dNTP, 0.5 Unit*Taq* enzyme in PCR buffer (10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin and 0.1% Triton X100) and 6 nM [a-^{32}P] dATP (Amersham). PCR parameters were 94°C for 30 sec, 40°C for 1 min, and 72°C for 30 sec for 40 cycles using a Cetus 9600 (Perkin-Elmer). Amplified and labeled cDNAs were separated on a 6% denaturing DNA sequencing gel. Gels were dried without fixation and bands were visualized by 16 hours of autoradiography using Kodak X-omatic film. Bands containing differentially expressed cDNA fragments of 150-450 nucleotides were cut out of the gel and DNA was extracted from the gel slices by electroelution onto DE-81 paper (Whatmann). After washing of the paper in low salt buffer (100 mM $LiCl_2$ in 10 mM TE buffer), and elution of the cDNA in high salt buffer (1 M $LiCl_2$ in 10 mM TE buffer with 20% ethanol) the cDNA was concentrated by precipitation in ethanol using glycogen as carrier. Reamplification of the cDNA fragments using the same PCR cycling parameters as described above but PCR buffer containing 2.5 mM of both the 10-mer and the anchor oligo and 100 mM dNTP. DE-81 paper allowed an efficient recovery of the DNA fragments and reamplification generated an average of 500 ng DNA after 40 cycles. Amplified PCR products were blunt-ended using the Klenow fragment of *E.coli* DNA Polymerase I (Pharmacia), purified on Sephacryl-S200 columns (Pharmacia), ligated into a SmaI linearized pBluescript vector II SK$^-$ (Stratagene) and transformed into *E.coli* using electroporation.

The last paragraph on page 26 has been amended as follows:

RT-PCR

Adult plant tissues from *Daucus carota* were obtained from S&G Seeds (Enkhuizen). Controlled pollination was performed by hand. Flower tissue RNA was obtained from three compete umbels for each time-point and contained all flower organs including pollen grains. 2 mg of total RNA from adult plant tissue or cell cultures was annealed at 42°C with 50 ng oligo (5'-

TCTTGGACCAGATAATTC-3' depicted as SEQ ID No. 14) in 10 ml annealing buffer (250 mM KCl, 10 mM Tris-HCl pH 8.3, 1 mM EDTA). After 30 min. annealing, 1 unit AMV-reverse transcriptase was added in a volume of 15 ml cDNA buffer (24 mM Tris-HCl pH 8.3, 16 mM $MgCl_2$, 8 mM DTT, 0.4 mM dNTP). The reverse transcription reaction took place for 90 min. at 42°C. PCR amplification of SERK-cDNA was carried out with two specific oligos for the SERK kinase domain, (5'-CTCTGATGACTTTCCAGTC-3') and (5'-AATGGCATTTGCATGG-3') (SEQ ID Nos. 15 and 16, respectively). Amplification was carried out with 30 cycles of 30 sec. at 94°C, annealing at 54°C for 30 sec. and extension at 72°C for 1 min., followed by a final extension for 10 min.at 72°C.

The Abstract has been amended as follows:

~~The present invention provides, *inter alia*, a method of producing apomictic seeds comprising the steps of:~~

~~(i) transforming plant material with a nucleotide sequence encoding a protein the presence of which in a cell, or membrane thereof, renders said cell embryogenic,~~

~~(ii) regenerating the thus transformed material into plants, or carpel containing parts thereof, and~~

~~(iii) expressing the sequence in the vicinity of the embryo sac.~~

~~The protein may be a leucine repeat rich receptor kinase which preferably is modified to the extent that the ligand binding domain is deleted or functionally inactivated.~~

A method and DNA sequence encoding a protein kinase for producing apomictic seeds, the method comprising the steps of transforming plant material with a nucleotide sequence encoding a protein the presence of which in a cell, or membrane thereof, renders the cell embryogenic, regenerating the thus transformed material into plants, or carpel containing parts thereof, and expressing the sequence in the vicinity of the embryo sac. The DNA sequence encodes a leucine repeat rich receptor kinase, which preferably is modified to the extent that the ligand-binding domain is deleted or functionally inactivated.

In the claims:

Claims 1-46 have been cancelled.

The following claims have been added:

47. An isolated DNA comprising a sequence encoding a protein kinase having the amino acid sequence depicted in SEQ ID No.3, SEQ ID No. 21 or SEQ ID No. 33, or a protein having an amino acid sequence which is at least 90% similar thereto and which hybridizes under stringent washing conditions of 3x20 min in 0.5% SSC, 1% SDS at 65° C with said isolated DNA having the sequence depicted in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 20, or SEQ ID No. 32 and encoding a protein kinase having the same activity as the sequences depicted in SEQ ID No. 3, SEQ ID No. 21, or SEQ ID No. 33.

48. The DNA according to claim 47, wherein the protein is a leucine rich repeat receptor like kinase and comprises a ligand binding domain, a proline box, a transmembrane domain, a kinase domain and a protein binding domain.

49. The DNA according to claim 47, wherein the protein is a leucine rich repeat receptor like kinase and comprises a ligand binding domain, a proline box, a transmembrane domain, a kinase domain and a protein binding domain.

50. The DNA according to claim 47, which further encodes a cell membrane targeting sequence.

51. The DNA according to claim 47, which further encodes a cell membrane targeting sequence.

52. The DNA according to claim 47, wherein the sequence is modified in that known mRNA instability motifs or polyadenylation signals are removed or codons which are preferred by the plant into which the DNA is to be inserted are used so that expression of the thus modified DNA in the said plant yields a protein having an amino acid sequence which is at least 90% similar to the sequence of that obtained by expression of the unmodified DNA in the organism in which the protein is endogenous.

53. The DNA according to claim 47, wherein the sequence is modified in that known mRNA instability motifs or polyadenylation signals are removed or codons which are preferred by the plant into which the DNA is to be inserted are used so that expression of the thus modified DNA in the said plant yields a protein having an amino acid sequence which is at least 90% similar to the sequence of that obtained by expression of the unmodified DNA in the organism in which the protein is endogenous.

54. An expression vector containing the DNA sequence as claimed in claim 47.

55. An expression vector containing the DNA sequence as claimed in claim 47.

56. An expression vector according to claim 54, in which the protein encoding region is under expression control of a developmentally regulated or inducible promoter.

57. An expression vector according to claim 55, in which the protein encoding region is under expression control of a developmentally regulated or inducible promoter.

58. An expression vector according to claim 56, wherein the promoter is one of the following: a promoter which regulates expression of SERK genes *in planta*, the carrot chitinase DcEP3-1 gene promoter, the *Arabidopsis* AtChitIV gene promoter, the *Arabidopsis* LTP-1 gene promoter, the *Arabidopsis* bel-1 gene promoter, the petunia fbp-7 gene promoter, the *Arabidopsis* ANT gene promoter, the promoter of the O126 gene from *Phalaenopsis*; the *Arabidopsis* DMC1 promoter, or the pTA7001 inducible promoter.

59. An expression vector according to claim 57, wherein the promoter is one of the following: a promoter which regulates expression of SERK genes *in planta*, the carrot chitinase DcEP3-1 gene promoter, the *Arabidopsis* AtChitIV gene promoter, the *Arabidopsis* LTP-1 gene promoter, the *Arabidopsis* bel-1 gene promoter, the petunia fbp-7 gene promoter, the *Arabidopsis* ANT gene promoter, the promoter of the O126 gene from *Phalaenopsis*; the *Arabidopsis* DMC1 promoter, or the pTA7001 inducible promoter.

60. A plant cell transformed with the vector of claim 54.

61. A plant cell transformed with the vector of claim 55.

62. Plant cell according to claim 60, which is part of a whole plant.

63. Plant cell according to claim 61, which is part of a whole plant.

64. Plants transformed with the vector of claim 54, or the seeds or progeny of such plants, wherein said seeds or progeny contain said vector of claim 54.

65. Plants transformed with the vector of claim 55, or the seeds or progeny of such plants, wherein said seeds or progeny contain said vector of claim 55.